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# Amygdala activation and its functional connectivity during perception of emotional faces in social phobia and panic disorder



L.R. Demenescu<sup>a,b,\*</sup>, R. KorteKaas<sup>b</sup>, H.R. Cremers<sup>c,d</sup>, R.J. Renken<sup>b</sup>, M.J. van Tol<sup>b,e</sup>,  
N.J.A. van der Wee<sup>f,g</sup>, D.J. Veltman<sup>h</sup>, J.A. den Boer<sup>i</sup>, K. Roelofs<sup>c</sup>, A. Aleman<sup>b,j</sup>

<sup>a</sup> Department of Psychiatry, Psychotherapy and Psychosomatics, University Hospital Aachen, RWTH Aachen University, Germany

<sup>b</sup> Department of Neuroscience, Neuroimaging Center, University Medical Center Groningen, University of Groningen, The Netherlands

<sup>c</sup> Radboud University Nijmegen, Behavioural Science Institute (BSI), Nijmegen, The Netherlands

<sup>d</sup> Department of Psychiatry, The University of Chicago, Chicago, IL, USA

<sup>e</sup> Leibniz Institute for Neurobiology, Otto von Guericke University, Magdeburg, Germany

<sup>f</sup> Department of Psychiatry, Leiden University Medical Center, The Netherlands

<sup>g</sup> Leiden Institute for Brain and Cognition, Leiden University Medical Center, The Netherlands

<sup>h</sup> Department of Psychiatry, Amsterdam VU University Medical Center, The Netherlands

<sup>i</sup> Department of Psychiatry, University Medical Center Groningen, The Netherlands

<sup>j</sup> Department of Psychology, University of Groningen, The Netherlands

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## ABSTRACT

Social phobia (SP) and panic disorder (PD) have been associated with aberrant amygdala responses to threat-related stimuli. The aim of the present study was to examine amygdala function and its connectivity with medial prefrontal cortex (mPFC) during emotional face perception in PD and SP, and the role of illness severity. Blood oxygen level dependent responses while perceiving emotional facial expressions were compared in 14 patients with PD, 17 patients with SP, 8 patients with comorbid PD and SP, and 16 healthy controls. We found that PD, but not SP, was associated with amygdala and lingual gyrus hypoactivation during perception of angry, fearful, happy and neutral faces, compared to healthy participants. No significant effect of PD and SP diagnoses was found on amygdala–mPFC connectivity. A positive correlation of anxiety symptom severity was found on amygdala–dorsal anterior cingulate and dorsal mPFC connectivity during perception of fearful faces. Amygdala hypoactivation suggests reduced responsiveness to positive and negative emotional faces in PD. Symptom severity, but not the presence of PD and SP diagnosis per se, explains most of the abnormalities in amygdala–mPFC connectivity during perception of fearful faces.

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## 1. Introduction

Social phobia (SP) has a prevalence of 12.1%, panic disorder (PD) of 4.7% (Kessler et al., 2005) and they frequently co-occur (Schneier et al., 1992). These anxiety disorders are characterized by an “alarm response [that normally occurs in response] to present or imminent danger” (Craske et al., 2009). However a clear distinction exists between SP and PD (Mannuzza et al., 1990). SP is characterized by extreme fear of negative evaluation and a resulting avoidance of

social or performance situations. PD is characterized by recurrent, spontaneous panic attacks which are acute episodes of intense fear accompanied by physical as well as cognitive symptoms (American Psychiatric Association [DSM-IV], 2000). Moreover, given the low recovery rate of SP (Beard et al., 2010) and the favorable improvement rate, but frequent recurrence, for PD (Francis et al., 2007), understanding the neural networks associated with these disorders is of importance.

As a key brain structure involved in processing threat-related stimuli (LeDoux, 2000), the amygdala is likely to play a role in the aforementioned anxiety disorders. Neuroimaging studies in subjects with SP using faces reported amygdala hyperactivation in response to angry (Stein et al., 2002), fearful (Campbell et al., 2007), happy (Straube et al., 2005) and neutral (Cooney et al., 2006; Birbaumer et al., 1998) faces. With regard to PD, Kent and Rauch (2003) in their review hypothesized that PD might be

\* Corresponding author. Department of Psychiatry, Psychotherapy and Psychosomatics, University Hospital Aachen, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany. Tel.: +49 (0)241 80 89413; fax: +49 (0)241 80 82401.

E-mail addresses: [ldemenescu@ukaachen.de](mailto:ldemenescu@ukaachen.de), [ramonademenescu@gmail.com](mailto:ramonademenescu@gmail.com) (L.R. Demenescu).

associated with abnormal amygdala function. In support of this hypothesis, amygdala hyperactivity has been reported during emotional conflict (Chechko et al., 2009) and during spontaneous panic attacks (Pfleiderer et al., 2007; Dresler et al., 2011) in PD. On the other hand, amygdala hypoactivation has been reported during anticipatory anxiety (Boshuisen et al., 2002) and in response to fearful faces (Pillay et al., 2006) in PD patients compared to healthy volunteers. The limited number of studies and the diversity of tasks make it difficult to draw a conclusion on the neural mechanism of emotional processing associated with PD. However, based on the existing literature it can be suggested that amygdala dysfunction is a key element of a shared mechanism involved in PD and SP.

Furthermore, abnormal neural responses in anxiety disorders have been reported in brain areas involved in attention and processing of facial features (prefrontal areas and fusiform gyrus [Gentili et al., 2008]), and emotional experience (insula [Straube et al., 2004; Etkin and Wager, 2007; Amir et al., 2005]). Anterior cingulate cortex (ACC) hyperactivation to disgusted faces has been reported in SP (Amir et al., 2005). In PD, aberrant ACC activation was reported in response to anticipatory anxiety, at rest (Boshuisen et al., 2002) and during an emotional conflict task (Chechko et al., 2009; van den Heuvel et al., 2005). From the above mentioned studies, we might conclude that not only the amygdala plays an important role in anxiety disorders, but that several other regions, including frontal areas, are also part of the neural mechanism associated with anxiety disorders.

Disrupted connectivity between amygdala and medial prefrontal cortex (mPFC) including ACC has been related to anxiety (Kim et al., 2011). Moreover, anxiety was associated with a negative amygdala–ventral mPFC connectivity, suggesting impaired emotion regulation, and a positive amygdala–dorsal mPFC connectivity, indicating hypervigilance to external stimuli (Straube et al., 2009). In addition, SP has been associated with a distinct right-sided abnormal functional connectivity between amygdala and superior temporal cortex, inferior parietal and middle PFC (Danti et al., 2010). In PD amygdala functional connectivity with brain areas involved in emotional processing is not clearly delineated. Gorman et al. (2000) in their review proposed that the “fear network”, involving amygdala as a main center and its interaction with mPFC, is central to PD.

The present study takes a step forward in understanding the neural mechanism of emotional processing associated with SP and PD, by not only looking at the individual brain areas, but also at their interaction. In particular, the present study aims to examine the pattern of brain activation and the coupling of amygdala response to mPFC regions during emotional face perception as an effect of SP and PD diagnoses. Additionally, we examined the extent to which the abnormalities in the neural network involved in emotional processing are related to anxiety severity rather than diagnosis-related factors. Moreover, by including not only patients with a diagnosis of PD or SP only, but also patients with PD and SP comorbidity, we aimed to determine whether there is a diagnosis-independent neural mechanism of disturbed emotional processing across anxiety disorders. Based on the literature, we hypothesized elevated amygdala, ACC and insula activation in response to angry, fearful, happy and neutral faces in SP. In PD abnormal amygdala activation was hypothesized to be limited to perception of fearful faces. Additionally, we expected abnormal coupling of amygdala–mPFC including the ACC during perception of emotional faces to be a common factor in anxiety disorders. For patients, we hypothesized that anxiety symptom severity would modulate amygdala response and its functional connectivity during perception of negative emotional faces.

## 2. Materials and methods

### 2.1. Participants

Participants were selected from the database of the Netherlands Study of Depression and Anxiety (NESDA, Penninx et al., 2008). Three centers participated: University Medical Center Groningen (UMCG), Amsterdam Medical Center (AMC) and Leiden University Medical Center (LUMC). The Ethical Review Board of each center approved the study. Inclusion criteria were: 1) age range 18–57 years, 2) no history of seizures or brain injury, 3) no criteria for any DSM axis I disorders other than social phobia (SP) and panic disorder with or without Agoraphobia (PD) (generalized anxiety disorder [GAD] was not an exclusion criterion), 4) no substance abuse, 5) no physical limitations that prohibited them from undergoing an fMRI examination and 6) no use of antidepressant or anxiolytic medication. Although in the NESDA fMRI study the use of selective serotonin reuptake inhibitors and infrequent benzodiazepine use (three times a week or within 48 h before the scanning) was not an exclusion criterion, in the present analyses these patients were excluded ( $n = 18$  patients), to exclude the confounding effects of medication. All subjects were native Dutch speakers. After receiving written information, each participant gave written informed consent.

Seventeen patients diagnosed with SP, fourteen patients diagnosed with PD and eight patients with a double diagnosis – SP + PD – were included in the present study. The diagnosis was established by trained clinical staff on the basis of the Composite International Diagnosis Interview (CIDI) – lifetime version 2.1 (Andrews and Peters, 1998) in accordance with DSM-IV criteria (American Psychiatric Association [DSM-IV], 2000). All patients met the criteria for primary SP and/or PD. Sixteen healthy controls (HC) were selected from 65 HC included in the whole NESDA MRI study (Demenescu et al., 2011; van Tol et al., 2011a,b) and this group was matched on sample size, age, educational level and gender. Healthy controls did not meet the criteria for any current Axis I disorder and had no history of psychiatric disorders.

Before the scanning session, all participants were evaluated by means of a battery of standardized questionnaires and structured interviews. Beck Anxiety Inventory (BAI, Beck et al., 1988), Fear Questionnaire (FQ, Marks and Mathews, 1979) and Montgomery–Åsberg Depression Rating Scale (MADRS, Montgomery and Åsberg, 1979) were administered to all participants.

### 2.2. Stimuli and paradigm

A detailed description of the paradigm has been published elsewhere (Demenescu et al., 2011). Briefly, the paradigm consisted of 120 color photographs of angry, fearful, sad, happy and neutral faces and 80 scrambled faces (control condition). The photographs were selected from the Karolinska Directed Emotional Faces System (Lundqvist et al., 1998). Twenty-four photographs were presented for each facial expression of emotion depicted by twelve female and twelve male actors. Photographs were presented pseudorandomly against a black screen for 2.5 s with an inter-stimulus (black screen) interval varying between 0.5 and 1.5 s (Wolfensberger et al., 2008). Participants were instructed to indicate the actor's gender. During the scrambled faces, participants had to press the corresponding button as indicated on the screen, i.e., an arrow pointing left or right. The faces paradigm was part of a larger MRI exam and was preceded by a planning (van Tol et al., 2011a) and a memory (van Tol et al., 2011b) paradigm.

### 2.3. MRI data acquisition

Images were acquired with a Philips 3T MR-scanner. A sense-8 (UMCG and LUMC) or a sense-6 (AMC) head coil was used for radio frequency transmission and reception. For each participant a series of echo planar imaging (EPI) volumes – sensitive to the blood oxygenation level dependent effect – were obtained, entailing a T2\*-weighted gradient echo sequence (repetition time [TR] = 2300 ms, echo time [TE] = 28.0 ms at UMCG and TE = 30.0 ms at AMC and LUMC) using axial whole-brain acquisition, with an interleaved slice acquisition order. The EPI volumes had 39 slices at UMCG and 35 slices at AMC and LUMC (0 mm gap, 3 mm thickness). The matrix sizes were: 64 × 64 voxels at UMCG and 96 × 96 voxels at AMC and LUMC. The in-plane resolution was 3 × 3 mm at UMCG and 2.29 × 2.29 mm at AMC and LUMC. The images were acquired parallel to the anterior–posterior commissure plane. A T1-weighted anatomical MRI was also acquired for each subject (TR = 9 ms, TE = 3.5 ms, matrix size 256 × 256, voxel size 1 × 1 × 1 mm).

### 2.4. Data analysis

Analyses on clinical and demographic data were performed with SPSS v.16.0 (SPSS Inc., Chicago, IL, USA). In order to test for significant differences between groups on demographic and clinical data analyses of variance were conducted. Repeated measures analysis of covariance was conducted on the mean reaction time calculated as the differences between angry/fearful/happy/neutral minus scrambled faces, testing for an effect of group and including centers (dummy variables) as a nuisance factor. Testing for a group effect on the gender discrimination accuracy was conducted using generalized linear model (GLZ, binary probit model) employing Wald Chi-Square test. Post-hoc tests were conducted using Bonferroni correction in case a significant effect was identified.

Functional imaging data collected in all centers were pre-processed and analyzed using the statistical parametric mapping software package (SPM5, Wellcome Department of Imaging Neuroscience, University College London) implemented in Matlab v.7.1.0 (The MathWorks Inc., MA, USA). The EPI volumes were corrected for slice acquisition time, realigned to the first volume, normalized to standard stereotaxic space defined by the Montreal Neurological Institute template during which voxels were resliced to 3 × 3 × 3 mm. Finally, spatial smoothing was applied using an 8 mm full-width at half-maximum Gaussian kernel.

Low-frequency temporal noise was removed by applying a high-pass filter (cut-off 128 s) to the fMRI time series at each voxel. Significant hemodynamic changes were identified using a general linear model, where events were modeled by convolution with the canonical hemodynamic response function (HRF, Friston et al., 1995). For each subject, the following contrasts were computed: “angry > scrambled”, “fearful > scrambled”, “sad > scrambled”, “happy > scrambled”, “neutral > scrambled” and “faces > scrambled”. The sad condition was not included in the present study because no hypothesis regarding perception of sad faces in anxiety patients was formulated.

Random-effects group analyses were conducted on weighted contrasts generated at the single-subject level modeled in a four (faces) by two (groups) repeated measurement analysis of covariance, with centers (dummy variable) added as nuisance factor. To test for an effect of diagnosis of PD or SP, the following contrasts reflecting PD (PD-only and SP + PD comorbidity vs. HC and SP-only) and SP (SP-only and SP + PD comorbidity versus HC and PD-only) were defined. Analyzing the data with a two-level factor group aims to eliminate the overlap in variance for the comorbidity group. Additionally, we tested for a common anxiety diagnoses effect defined as all patients (SP-only, PD-only and SP + PD comorbidity)

versus HC. A whole brain voxel-wise analysis was performed together with region of interest (ROI) analyses corrected for multiple comparisons ( $p < 0.05$  Family-Wise Error [FWE]). Amygdala, ACC, mPFC and insula anatomical ROIs were defined using WFU Pickatlas (Maldjian et al., 2003).

Further, we tested how anxiety symptom severity modulates the neural response involved in emotional processing. To this end regression analyses were conducted on the contrast images: “angry > neutral”, “fearful > neutral” and “happy > neutral” across all patients using BAI score as a regressor of interest and centers as confound.

Psychophysiological interaction (PPI, Friston et al., 1997) analysis was conducted to examine the difference in amygdala–mPFC connectivity modulated by perception of emotional faces as a factor of independent and common PD and SP diagnoses. This interaction was examined for left and right amygdala defined as “seed” regions. Amygdala activation was identified at the subject level for the contrast “faces > scrambled” inspected at  $p < 0.05$  uncorrected. The deconvolved time series was extracted from a sphere of 5 mm radius centered around the peak activated voxel within left and right amygdala (defined by WFU Pickatlas) for each participant. This time-series reflects amygdala reactivity across all faces including neutral (>scrambled), i.e., we chose not to restrict the “seed” region to only those (amygdala) voxels that were activated in response to emotional faces. Choosing only voxels activated by the emotional faces could bias our results in finding a task related (emotion > neutral) connectivity pattern.

The PPI term was calculated as the element-by-element product of the amygdala time series and a vector coding for the task effect (“angry > neutral”, “fearful > neutral” and “happy > neutral”). This product was subsequently re-convolved with the HRF. The interaction term was entered as a regressor into a first level model together with the amygdala time series (physiological variable) and a vector coding for the task effect (psychological variable).

The individual contrast images were then entered into a second level analysis to identify the “target” regions showing changes in connectivity with the “seed” region depending on experimental context: angry, fearful or happy versus neutral faces perception (left and/or right) were included in these analyses. Thus, for left amygdala connectivity analysis 6 PD, 11 SP, 4 PD + SP and 13 HC were included whereas for right amygdala connectivity analysis 6 PD, 11 SP, 3 PD + SP and 14 HC could be included. Repeated measurement analysis of covariance (3 × 2) with centers defined as confound was conducted testing for independent- and common-anxiety diagnoses effects on amygdala connectivity. Small volume correction ( $p_{\text{FWE}} < 0.05$ ) was applied for the *a priori* defined areas, i.e., left and right: mPFC and ACC defined using WFU Pickatlas (Maldjian et al., 2003).

The impact of anxiety symptoms severity on amygdala–mPFC/ACC connectivity was assessed by simple regression analyses against the BAI scores across anxiety patients and controlling for a possible center effect. The regression analyses were conducted on the PPI individual contrast images: “angry > neutral”, “fearful > neutral” and “happy > neutral”.

## 3. Results

### 3.1. Sample characteristics and behavioral data

Table 1 displays the group characteristics. No significant group effect was found on age, years of education, gender and handedness (all  $p > 0.05$ ). A main effect of group was found on BAI ( $F[3,49] = 10.37$ ,  $p < 0.001$ ), FQ ( $F[3,51] = 10.07$ ,  $p < 0.001$ ) and MADRS ( $F[3,52] = 8.46$ ,  $p < 0.001$ ) scores. Post-hoc tests showed



**Table 1**

Characteristics of the groups and behavioral data (*n* represents the number of participants). Data are presented as mean (Standard Deviation) unless otherwise specified.

	HC ( <i>n</i> = 16)	PD ( <i>n</i> = 14)	SP ( <i>n</i> = 17)	SP + PD ( <i>n</i> = 8)
Age (SD)	33.56 (9.62)	33.07 (10.27)	36.12 (9.95)	31.38 (8.36)
Education years (SD)	13.44 (2.87)	13.64 (3.27)	12.88 (3.52)	12.50 (2.62)
Right-handed (%)	87.5	92.9	94.1	87.5
Male (%)	31.3	21.4	35.3	12.5
Center (AMC/LUMC/UMCG)	7/4/5	2/4/8	4/5/8	2/4/2
6 mos diag (%)	0.0//0.0//0.0//0.0	0.0//14.3//85.7//7.1	100.0//0.0//0.0//0.0	100.0//87.5//12.5//25.0
SP//PD w Ago//PD w/o Ago//GAD				
Lifetime (%)	0.0//0.0//0.0//0.0	7.1//14.3//85.7//14.3	100.0//5.9//0.0//17.6	100.0//87.5//12.5//25.0
SP//PD w Ago//PD w/o Ago//GAD				
Onset SP (SD)	0.0	10.0 (0.0)	15.94 (12.58)	16.50 (9.55)
Onset PD w Ago (SD)	0.0	15.00 (4.24)	29.00 (0.0)	17.86 (11.08)
Onset PD w/o Ago (SD)	0.0	22.67 (13.67)	0.0	10.00 (0.0)
Onset GAD (SD)	0.0	17.50 (2.12)	26.33 (9.24)	21.50 (16.26)
BAI (SD)	2.25 (2.38)	6.36 (21.16)	13.12 (10.48)	24.38 (10.79)*
FQ (SD)	8.25 (8.44)	24.55 (23.70)	32.88 (18.33)*	44.75 (15.54)*
MADRS (SD)	0.81 (1.42)	6.43 (12.75)	9.63 (9.33)*	15.25 (8.41)*
<b>Reaction time (milliseconds), mean (SD)</b>				
Angry vs. scrambled	61.49 (77.84)	68.56 (128.74)	87.10 (119.92)	77.88 (95.65)
Fearful vs. scrambled	116.61 (69.36)	45.28 (141.10)	105.86 (100.34)	86.05 (91.03)
Happy vs. scrambled	115.33 (69.55)	68.89 (103.81)	111.50 (96.98)	88.18 (89.06)
Neutral vs. scrambled	116.02 (45.94)	74.59 (125.62)	105.97 (109.88)	121.56 (87.41)
<b>Correct responses (%)</b>				
Angry	99.3	95.9	99.3	99.0
Fearful	99.0	95.8	98.8	97.1
Happy	98.5	94.9	98.3	97.6
Neutral	98.8	91.4	98.3	98.6
Baseline	98.3	96.7	94.2	99.1

Note: HC – healthy controls; PD – Panic Disorder; SP – Social Phobia; PD + SP – Panic disorder and Social Phobia comorbidity. AMC – Amsterdam Medical Center, LUMC – Leiden University Medical Center, UMCG – University Medical Center Groningen. 6 mos diag – diagnosis presence for the last 6 months. SP – Social Phobia, PD w Ago – Panic Disorder with Agoraphobia, PD w/o Ago – Panic Disorder without Agoraphobia, GAD – General Anxiety Disorder. Diagnosis Onset – age in years. BAI – Beck Anxiety Inventory. FQ – Fear Questionnaire. MADRS – Montgomery–Åsberg Depression Rating Scale. \*Significant difference between patient groups and healthy controls, all  $p < 0.05$ .

that patients with PD + SP comorbidity scored significantly higher on BAI, FQ and MADRS than HC (all  $p < 0.05$ ). SP-only patients scored significantly higher on FQ ( $p = 0.003$ ) and MADRS ( $p = 0.043$ ) compared to HC. No significant difference was found between PD-only patients and HC on BAI, FQ or MADRS score. Between patient groups only patients with PD + SP comorbidity scored significantly higher on BAI ( $p = 0.016$ ) and FQ ( $p = 0.008$ ) compared to PD-only.

The mean differences of the reaction time to angry/fearful/happy/neutral minus scrambled faces are displayed in Table 1. No significant effect of group was found ( $F[3,50] = 0.846$ ,  $p = 0.475$ ) on the reaction time. With regard to accuracy, a significant effect was found for group ( $\chi^2[3] = 50.29$ ,  $p < 0.005$ ), whereas no significant effect was found for emotion ( $\chi^2[3] = 5.01$ ,  $p = 0.171$ ) and group by emotion interaction ( $\chi^2[9] = 2.67$ ,  $p = 0.976$ ). Post-hoc tests showed that PD patients were prone to more errors than patients with SP and PD + SP comorbidity, and HC ( $p < 0.05$ ). Table 1 displays the percentage of correct responses for each condition within each group.

### 3.2. Imaging data

Perception of faces (>scrambled) elicited increased activation in bilateral fusiform gyrus (Brodmann area 37 left:  $[-42, -45, -21]$  and right:  $[42, -51, -24]$ ) and bilateral amygdala (left:  $[-18, -9, -15]$  and right:  $[21, -6, -15]$ ) for  $p_{FWE} < 0.05$ .

Regarding group differences in brain activation during perception of faces, a significant effect of PD diagnosis was observed in left amygdala ( $[-18, -3, -21]$ ,  $Z = 3.42$ ,  $p_{FWE} < 0.05$ , SVC). Outside our ROIs, an effect of PD diagnosis was observed in the right lingual gyrus ( $[36, -78, -15]$ ,  $Z = 4.84$ ,  $k = 25$ , whole brain corrected  $p_{FWE} < 0.05$ ). Post-hoc tests showed that decreased left amygdala and right lingual gyrus activation were found as a function of PD in response to angry, fearful, happy and neutral faces relative to HC

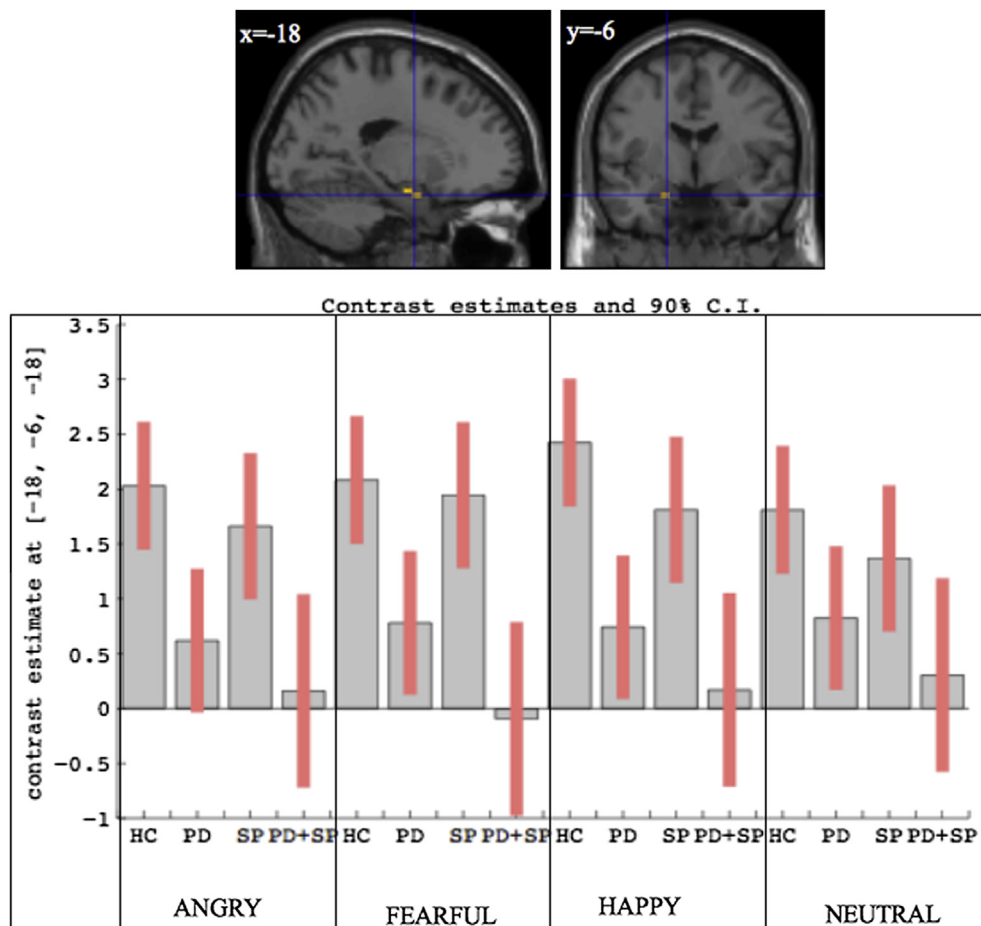
and SP (Fig. 1). No significant effect of SP diagnosis was found in the *a priori* defined areas or outside our ROIs in response to faces (>scrambled). The same was true when looking for a common-diagnoses effect, i.e., all patients versus HC: no significant effect of anxiety disorders was found. Additionally, we tested for an effect of group on emotion versus neutral contrasts. No significant effect was observed within or outside our ROIs.

Because some of the patients had a second diagnosis of GAD and because this might have had an influence on the neural response during perception of faces, we conducted an additional analysis. A one sample *t* test was conducted on faces (>scrambled) across all patients including GAD diagnosis as a regressor of interest. No significant effect of GAD diagnosis was observed in the neural response to faces in anxiety patients.

Regarding symptom severity a tendency toward a positive relation was found between right dACC ( $Z = 3.27$ ,  $p_{FWE} = 0.072$ , SVC) activation and BAI score during perception of fearful faces in patients with anxiety diagnoses. No other significant association was found between dmPFC or ACC and anxiety symptoms during perception of angry or happy (>neutral) faces.

### 3.3. Amygdala functional connectivity modulated by anxiety

No significant independent or common PD and SP diagnosis effects were found on left or right amygdala connectivity. However, an effect of symptom severity was observed on left amygdala connectivity during fearful (>neutral) faces perception in anxiety patients. This effect was not found in response to angry or happy (>neutral) emotions or in HC. Thus, a positive correlation was found between anxiety symptom severity (indexed by BAI) and left amygdala–right rostral ACC (rACC,  $[0, 39, 15]$   $p_{FWE} < 0.05$ , SVC, Fig. 2A) coupling and left amygdala–left dorsal mPFC (dmPFC,  $[-9, 36, 36]$ , Fig. 2B) coupling during perception of fearful faces in patients. In other words, the strength of the connectivity between



**Fig. 1.** Left amygdala peak response  $[-18, -6, -18]$  to angry, fearful, happy and neutral faces ( $>$ scrambled) as an effect of PD diagnosis ( $p_{FWE} < 0.05$ , small volume correction). The bars indicate the mean % signal change in the left amygdala within each group during perception of angry, fearful, happy and neutral faces ( $>$ scrambled). The red lines indicate the 90% confidence interval (C.I.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

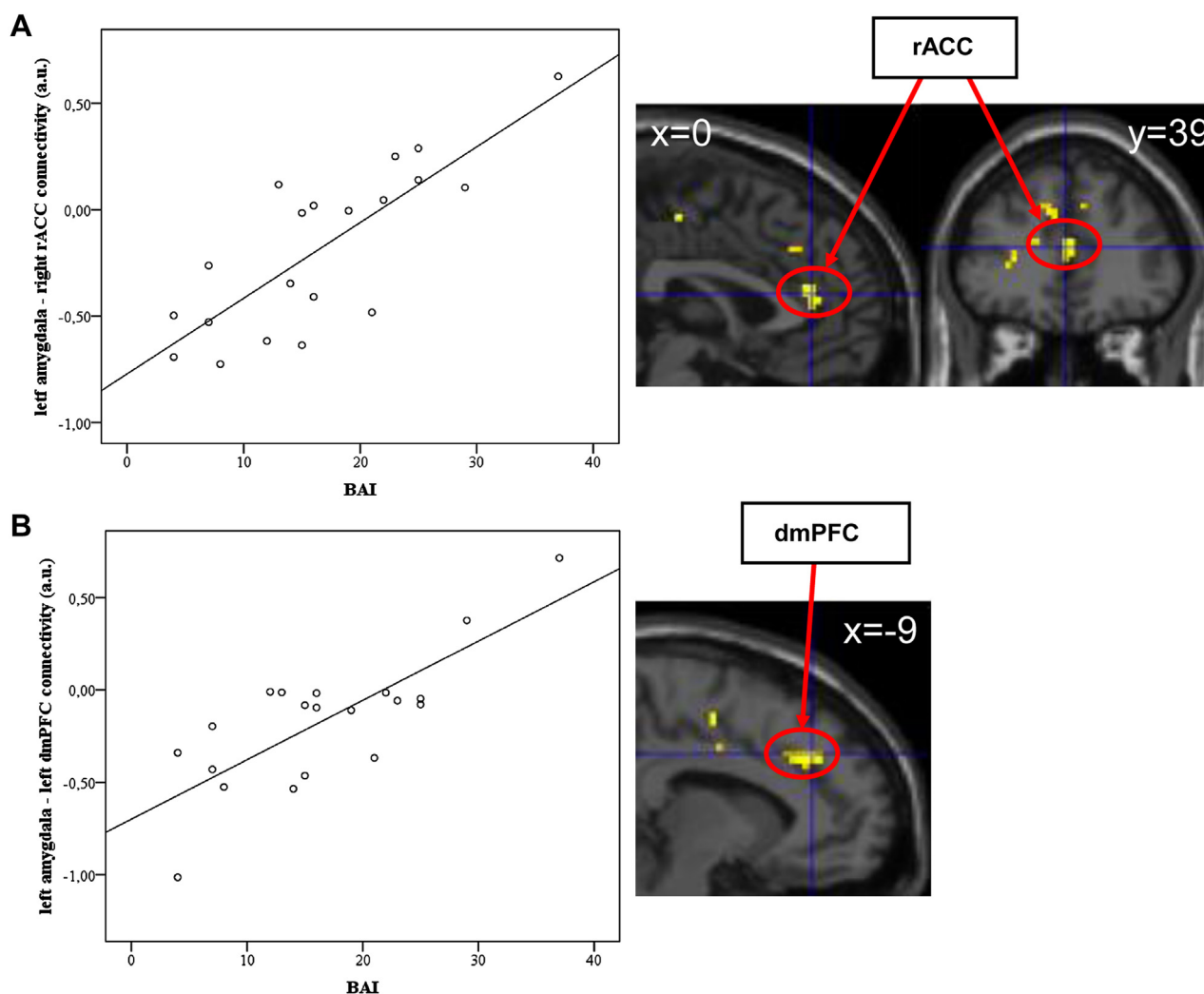
left amygdala and rACC/dmPFC correlated positively with symptom severity during perception of fearful faces.

#### 4. Discussion

In the present study we investigated regional brain activation and functional connectivity during perception of emotional faces in relation to independent and common SP and PD diagnoses. We expected abnormal activation in areas involved in emotional face processing, *i.e.*, amygdala, ACC, insula and abnormal amygdala–mPFC (including ACC) connectivity during processing of emotional faces as a factor of SP and PD diagnoses. We found blunted left amygdala and right lingual gyrus activation to faces as an effect of PD, relative to HC and SP. In contrast to our expectation, no effect of SP was observed on amygdala response to faces. Also, no significant effect of common PD and SP diagnoses was found on amygdala response relative to HC. Likewise, no significant effect of independent or common PD and SP diagnoses was found on the amygdala–mPFC/ACC connectivity during perception of emotional versus neutral faces. Notwithstanding, a modulatory effect of anxiety symptom severity was observed on left amygdala–rACC/dmPFC connectivity across patients with SP and PD but not in HC. Thus, the present findings suggest a diagnosis-specific effect on regional brain activation, but not on amygdala–mPFC/ACC connectivity during perception of emotional faces. Hence, amygdala–dmPFC/rACC connectivity is predicted by anxiety symptom severity in anxiety patients.

Amygdala hypoactivation was observed in response to withdrawal (fearful), approach (angry and happy) emotions and neutral faces in PD patients compared to HC. Evidence of amygdala hypoactivation to fearful faces in PD relative to HC has been previously reported suggesting a diminished emotional response during perception of fearful faces (Pillay et al., 2006). Additionally, amygdala hypoactivation was also reported during anticipatory anxiety in PD patients compared to HC, suggesting that PD is associated with amygdala functional impairment during anticipatory anxiety (Boshuisen et al., 2002). Nevertheless, aberrant amygdala response in PD was reported during an emotional conflict task (Chechko et al., 2009) and to panic-related words (van den Heuvel et al., 2005). These inconsistencies in findings may be explained by different levels of paradigm difficulties, fear-related stimuli, but also by hidden genetic differences. For example, Domschke and Dannlowski (2010) in their review suggest that 5-HT1A polymorphisms and genetic variation in the noradrenergic and dopaminergic system drive a distorted processing of threat-related stimuli.

Besides amygdala hypoactivity, we found an effect of PD diagnosis in right lingual gyrus. The involvement of lingual gyrus in face perception has been previously reported (Puce et al., 1996). It should be mentioned that this pattern of abnormal activation was specific to PD-only, because we controlled for an effect of SP diagnosis. The present findings add support to the existing literature suggesting that PD is associated with abnormal emotional responses not only to fearful but also to angry, happy and neutral faces.



**Fig. 2.** Left amygdala connectivity correlates with anxiety symptom severity during perception of fearful (>neutral) faces in anxiety patients. A. Positive association between rostral ACC (rACC)–left amygdala connectivity during fearful (>neutral) faces and BAI score. B. Positive association between left dmPFC–amygdala connectivity during fearful (>neutral) faces and BAI score.

In contrast to our hypothesis of abnormal amygdala, ACC and insula responsiveness to faces in SP, no significant effect of SP diagnosis was found within these areas involved in emotional processing. SP patients compared to HC and PD showed no significant differences in amygdala, ACC and insula response to faces versus a low baseline (scrambled faces) or in response to emotional versus neutral faces. In line with the present finding, Furmark et al. (2009) did not report amygdala hyperactivation in SP compared to HC. Furthermore, they showed that aberrant amygdala response is related to serotonergic polymorphisms rather than to social anxiety diagnosis (Furmark et al., 2009). Thus, it might be that the lack of significant amygdala response difference between SP and HC can be explained by genetic variability, although this was not investigated in the current study. Contrary to these findings, other studies employing a block design in SP patients did report amygdala hyperactivation when processing negative (Stein et al., 2002; Campbell et al., 2007; Straube et al., 2004), positive (Straube et al., 2005) or neutral (Cooney et al., 2006; Birbaumer et al., 1998; Gentili et al., 2008) faces. Moreover, Yoon et al. (2007) reported amygdala hyperactivation to high versus low emotional intensity in faces in SP compared to HC suggesting that amygdala reactivity is modulated by emotional intensity. Discrepancies between the present and previous studies might be explained by design, *i.e.*, event-

related for the present study versus block design in others and emotional intensity of facial expressions (Yoon et al., 2007). Thus, it might be that a longer exposure time to highly emotional stimuli leads to an aberrant emotional response in SP.

Also in contrast to our expectations, no effects of independent or common-anxiety diagnoses were found on amygdala–mPFC functional connectivity during emotional faces perception. Nevertheless, a positive relation was observed between anxiety symptom severity and left amygdala–rACC/dmPFC connectivity during perception of fearful versus neutral faces. In other words, amygdala–rACC and amygdala–dmPFC connectivity appears to be stronger when anxiety symptoms are more severe and this effect was limited to fearful faces. Withal, this modulatory effect of anxiety symptom severity was restrained to amygdala connectivity and not to regional brain activation to fearful (>neutral) faces. Angry and fearful faces are both considered threat-related stimuli. However, angry faces represent a more direct form of threat, whereas fearful faces signal the presence of undetermined threat, *i.e.*, the source of threat, danger is more ambiguous (Whalen, 1998). Ewbank et al. (2009) showed that increased anxiety level is associated with a stronger amygdala response to unattended fearful faces relative to angry faces. This indicates that the two emotions represent “distinct forms of threat”. Additionally to amygdala,

dorsal ACC activation to strong threat stimuli has been reported to be modulated by anxiety level (Straube et al., 2009), suggesting a role of dorsal ACC in anticipatory anxiety and hypervigilance to environmental stimuli (Straube et al., 2009, 2007). It might be concluded that an increased anxiety level is associated with an elevated emotional response whereas the hypervigilance toward threat-related stimuli is associated with an ambiguous source of threat. Undetermined, ambiguous, signals of threat may be of more relevance to SP than directed threat as in the case of anger, as the context of SP typically does not involve clearly defined forms of danger, but rather a vague sense of threat.

Abnormal amygdala–mPFC/ACC connectivity has been previously reported in association with anxiety (Etkin and Wager, 2007). Åhs et al. (2009) reported a negative covariation between amygdala and supragenual ACC rCBF during fear-relevant but non-phobic conditions in sixteen females with a diagnosis of specific phobia suggesting a “phobia-related functional decoupling”. Stronger amygdala–dmPFC connectivity has been reported during self-directed criticism in patients with generalized social phobia compared to HC suggesting the maintenance of “negative self-referential evaluation” (Blair et al., 2008a). It is usually thought that the dorsal mPFC/ACC is cognition-related, whereas the ventral mPFC/ACC is the affective division (Bush et al., 2000). In contrast, a recent review concluded that dorsal mPFC/ACC function is associated with expression of fear (Milad et al., 2007) and appraisal, whereas ventral mPFC/ACC has an inhibitory role on negative emotions (Etkin et al., 2011). The authors concluded that the amygdala–dorsal mPFC/ACC connectivity is associated with “negative emotion generation” and that the amygdala–ventral mPFC/ACC connectivity has an inhibitory function (Etkin et al., 2011).

Considering the above mentioned findings, and in conjunction with our results, it might be concluded that a more pronounced anxiety severity is associated with an elevated negative emotional response and hypervigilance. This is limited, though, to perception of fearful faces when the threat source is ambiguous. It should be noted here that anxiety severity was not associated with abnormal neuronal activation in specific brain areas to fearful or any other emotional versus neutral faces. The association was only specific to the dynamic connectivity among brain areas involved in emotional processing. Overall we might conclude that anxiety severity correlates with connectivity among brain areas, whereas specific anxiety diagnosis, i.e., PD is associated with abnormal regional brain activation.

One limitation of the present study is the relatively small number of patients that were included for the separate diagnostic categories. Further studies involving larger samples are needed. Another limitation might be the presence of GAD (Blair et al., 2008b) as a secondary diagnosis for some of the anxiety patients. However, we did test for an effect of GAD and no significant effect was observed.

To summarize, this study provides evidence for a differential effect of anxiety diagnosis on the amygdala response to faces. Specifically, PD, but not SP or comorbid-anxiety diagnoses, was found to be associated with a reduced amygdala activation to angry, fearful, happy and neutral faces. Nevertheless, abnormal amygdala–rACC/dmPFC connectivity was a function of anxiety symptoms severity in patients. Additionally, it might be concluded that the comorbidity of PD and SP diagnoses could not be conceptualized as a summation of these diagnoses.

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#### Contributors

Liliana R. Demenescu, Marie-Jose Van Tol, André Aleman, Nic J.A. van der Wee, Dick J. Veltman designed the study and were involved in data collection and data analysis. Rudie Kortekaas, Henk R. Cremers, Karin Roelofs and Remco J. Renken helped with data analysis. Liliana R. Demenescu wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

#### Conflict of interest

L.R. Demenescu, R. Kortekaas, H.R. Cremers, M.J. van Tol, N.J.A. van der Wee, D.J. Veltman, J.A. den Boer, K. Roelofs report no financial interests or potential conflicts of interest. R. Renken declares to have participated in commercial projects from the clinical research organizations Xendo and PRA International since 2009. A. Aleman received an investigator-initiated unrestricted research grant from Bristol-Myers Squibb and speakers honoraria from AstraZeneca, Bristol-Myers Squibb, GlaxoSmithKline.

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